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| (54) Title: HEALTH BEVERAGE CONTAINING THE EXTRACT OF <i>PHELLINUS LINTEUS</i> | | | |
| (57) Abstract According to the present invention, a health beverage containing the extract of <i>Phellinus linteus</i> as main ingredient and manufacturing method thereof are disclosed and regular drinking of these beverages can reduce the incidence rate of cancers. | | | |

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Health beverage containing the extract of *Phellinus linteus*

Detailed Description of the invention

The present invention concerns with a health beverage containing the 5 extract of *Phellinus linteus* as main ingredient and manufacturing method thereof.

Basidiomycetes have been used for a long period of time as an oriental folk herbal medicine. Especially, the basidiomycetes-produced polysaccharides were known to have an excellent anticancer activity with 10 less side effects (toxicologically safe) and an activity of potentiating the immune system. Two methods were known for the use of basidiomycetes: 1) The use of fruiting body of basidiomycetes by collecting directly from natural resources or planting the fruiting body and 2) The use of mycelia after separating mycelia from fruiting body 15. and cultivating the mycelia. The method of directly collecting naturally formed fruiting body causes the depletion of natural resources and ecological disruption. Therefore, artificially cultivating method has been employed, however, this method has some disadvantages; 1) slow growing rate, and 2) bacterial contamination.

20 In order to solve the above mentioned problems, the inventors developed the method for cultivating mycelia of *Phellinus linteus* after a long-term study and obtained patents or are applying for patents (Korea

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Patent Publication No. 92-1367, Polysaccharides and a method of their production; Korea Patent Publication No. 92-1194, Manufacturing method of mycelia of *Phellinus linteus*; Korea Patent Publication No. 97-9150, Artificial liquid cultivation of mycelia of *Phellinus linteus* and 5 manufacturing method of compounds having anticancer-immune activity; Korea Patent Laid Open Publication No. 97-15743, Manufacturing method of polysaccharides having anticancer-immune activity isolated from *Phellinus linteus*; and Korea Patent Laid Open Publication No. 97-1531, A new strain of *Phellinus linteus* producing anticancer-immune activity).

10 The polysaccharides with potentiating immune system could be obtained after purification of the extract of *Phellinus linteus* (Korea Patent Publication No. 92-1367; Korea Patent Laid Open Publication No. 97-15743), and this could be used in this invention.

The extract and polysaccharides ("*Phellinus linteus* extract") 15 obtained from mycelia of *Phellinus linteus* by above mentioned method have the following pharmacological activities; 1) potentiation of immune system when combination chemotherapy was employed to treat gastrointestinal cancer[The New Korean Medical Journal, Vol. 39, No. 11, November , 1996] and hepatoma, and after cancer dissection 20 surgery[Journal of Korean Cancer Association, Vol.29, No. 3, June,1997 and 2) detoxification activities. Therefore, the "*Phellinus linteus* extract" has been used as a medicine in the form of powder or capsule.

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Since the modern population are exposed to a variety of air pollutants and carcinogenic compounds, the incidence rate of cancer is very high. Not only the cure rate of cancer is low, but also the cancer patients are suffering from severe pain and survival rate is not high, the best 5 method is to prevent cancer.

Now, the present inventors developed health beverages containing the extract of *Phellinus linteus* as main ingredient, after long-time study. Regular drinking of these beverages can reduce the incidence rate of cancer and the taste and color of these beverages are nearly the 10 same with those of common beverages, and no precipitates are formed.

The present invention covers the following beverages containing the extract of *Phellinus linteus*; fruit juices, alcoholics, coke, milk, coffee, tea, and common alcoholic and non-alcoholic beverages.

The juices contain orange juice, tomato juice, apple juice, strawberry 15 juice, pear juice, and other common fruit juices. The concentrated (100%) and diluted (by adding water) juices are also covered by the present invention.

The coffees contain dried pre-mix and canned or bottled instant coffee.

20 The teas contain dried pre-mix and caned or bottled instant tea of green tea and red tea.

The present invention also covers flavored beverages. The flavored

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beverages are nonalcoholic beverages containing orange-, grape-, lemon-, and strawberry-flavored beverages.

The present invention also covers alcoholic beverages; beers and wines, and apple- and lemon-containing alcoholic beverages, and other 5 low-alcoholic beverages.

1-5,000 mg (preferably 5-1,000 mg) of the extract of *Phellinus linteus* could be added to 100 ml of beverages.

1 - 5,000mg (preferably 5 - 1,000mg) of the extract of *Phellinus linteus* could be added to 1,000mg of dried pre-mix of coffee or tea.

10 The present healthy beverages where the extract of *Phellinus linteus* was added to common beverages could be sterilized, if necessary.

The following examples and experiments explain concisely the present invention.

15

Example 1.

Culture of *Phellinus linteus* strain, FERM BP-2639(Deposited to Japan Institute of Microorganism).

The 1 litter of culture medium containing 50 g of glucose, 10 g of 20 pepton, 10 g of yeast extract solution, and 0.5 g K₂HPO₄ was employed to culture the *Phellinus linteus* strain. The 1 litter of main culture medium containing 10 g of soluble starch, 50 g of glucose, 5 g of yeast

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extract solution, 10 g of corn steep powder, 0.5 g of K_2HPO_4 , 0.3 g of $MgSO_4$, and trace elements of $MnCl_2$, $FeSO_4$, and $ZnSO_4$ was used. Both the seed culture medium and main culture medium were adjusted to pH 5.0 before sterilization.

5 After sterilization of 500 ml flask containing 50 ml of seed culture medium for 20 min at 121 °C, *Phellinus linteus* strain, FERM BP -2639[Deposited to Japan Institute of Microorganism] on the potato-glucose-agar slant was collected with small portion of the slant and finally cultured with agitation for 3 days at 28 °C ("the first seed culture medium"). The jar fermenter containing 3l of seed culture medium was sterilized for 30 min at 121 °C and 50 ml of the above mentioned ("the first seed culture") medium was added to the jar fermenter, and then were cultured for 3 days at 28 °C ("the second seed culture medium").

15 6 litter of "the second seed culture medium" was added to 500 litter of presterilized fermenter containing 300 litter of main culture medium and the mixture was cultured with agitation at 28 °C with 300 l/min of aeration and 100-200 rpm of stirring. The above mixture was added to a presterilized fermenter containing 3000 litter of main culture medium and 20 the mixture was cultured for 3 days with 3000 l/min of aeration and 100-200 rpm of stirring. The above procedures were repeated to obtain 25 g/l of mycelia (as a dry weight) at 3 days after inoculation.

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Example 2

Extraction of the compounds having the activities of anticancer and potentiating immune system. The mycelia obtained from example 1 were extracted and purified by the following procedures. 500 ml of 5 distilled water were added to 100 g of the mycelia and extracted twice for 2 h of boiling at 90-100 °C. After discarding the cake, the extracted water layer was collected. The extracted water layer was concentrated to 100 ml under vacuum and 3 volumes of 95% ethanol were added.

After overnight standing, centrifugation was performed for 30 min at 10 3000 rpm. 100 ml of water was added to dissolve the precipitate and the mycelia having molecular weight of less than 8000 was obtained using semipermeable membrane for 3 days. The resultant high molecular weight component containing polysaccharides, 3 g, was obtained after freeze drying at -70 °C.

15

Example 3.

Extraction of crude polysaccharides

10-20 volumes of water were added to the culture medium obtained from example 1 and the mixture was boiled for 1-2 hr at 50-100 °C. 20 After centrifugation, the filtrate was concentrated using Reduced-pressure Film Evaporator. Crude polysaccharides were obtained either freeze drying or spray drying from the above mentioned

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concentrated solution.

Experiment 1.

Acute toxicity test

5 The extract of *Phellinus linteus* obtained from example 2, 10,000 mg/kg, was injected intraperitoneally to 4-week old ICR mouse (n=10). These were no significant findings after 14 days.

Experiment 2.

10 Anticancer activity

The anticancer activity against sarcoma 180 of the extract of *Phellinus linteus* obtained from example 2 was performed in 4-week-old ICR mice (n=10). At seventh day after implantation of sarcoma 180 to ICR mice peritoneum, 1×10^6 cells were implanted subcutaneously into 15 the mice left groin. The extract of *Phellinus linteus* was dissolved in normal saline injectable solution and filtered using 0.45 μm filter. And then 100 mg/kg was injected intraperitoneally everyday for 10 days from 24 h after implantation into the groin. At 30th day after implantation into the groin, the cancer mass was isolated and the percentages of 20 cancer growth inhibition were measured. The results are listed in Table 1. As listed in Table 1, the anticancer activity of the extract of *Phellinus linteus* was approximately 71.5%.

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Table 1. Anticancer activity of the extract of *Phellinus linteus* against sarcoma 180

| Treatment | Number of animal | Weight of sarcoma 180 (g) | Inhibition(%) |
|--|------------------|---------------------------|---------------|
| Control | 8 | 2.00±0.58 | - |
| Treated with the extract of <i>Phellinus linteus</i> (100 mg/kg, ip) | 8 | 0.57±0.93 (p<0.01) | 71.5 |

10 It has been found that the extract of *Phellinus linteus* had an excellent activity against gastrointestinal cancer (New Med. J. Korea, 39, 1996) and had potentiating immune system (J. Kor. Cancer Assoc., 29, 1997), and had an excellent effect against B16 (Int. J. Immunopharmacol., 18, 295-303, 1996)

15 Above data indicates that the extract of *Phellinus linteus* has little toxicity and has anticancer activity. Therefore, the extract could be used as health food to prevent and treat the cancer

20 The present invention will be illustrated in detail by the preparation examples below.

Preparation Example 1

To each 100ml of undiluted orange juice was added each 10mg,

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20mg, 30mg, 40mg, 50mg and 100mg of extract of *Phellinus linteus*(PL Ex.) and each mixture was dissolved with well stirring at ambient temperature and filled in each bottle of 100ml and sterilized. The color, taste and precipitate of each orange juice prepared was compared with 5 those of original orange juice.

The results are as follows.

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

Preparation Example 2

To each 100ml of milk was added each 10mg, 20mg, 30mg, 40mg, 15 50mg and 100mg of extract of *Phellinus linteus*(PL Ex.). Each mixture was dissolved with well stirring at ambient temperature, filled in each bottle of 100ml and was sterilized. The color, taste and precipitate of each milk prepared was compared with those of original milk.

The results are as follows.

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

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Preparation Example 3

To each undiluted cola liquid was added each 10mg, 20mg, 30mg, 40mg, 50mg and 100mg of extract of *Phellinus linteus*(PL Ex.). Each mixture was diluted with purified water, controlled sugar content with 5 sugar and filled in each bottle of 100ml. CO₂ gas was filled in each mixture and was sealed. Each mixture was sterilized in a conventional preparation method of cola to prepare each cola. The color, taste and precipitate of each cola prepared was compared with those of original cola.

10

The results are as follows.

15

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

Preparation example 4

To each 100ml of beer was added 10mg, 20mg, 30mg, 40mg, 50mg and 100mg of extract of *Phellinus linteus*(PL Ex.) and each mixture 20 was dissolved with well stirring and filled in each bottle of 100ml. To the mixture was filled CO₂ gas to be controlled to the conventional CO₂ gas pressure of beer and was sterilized in a conventional preparation

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method of beer. The color, taste and precipitate of each beer prepared was compared with those of original beer.

The results are as follows.

5

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

10 Preparation Example 5

To each 100ml of portwine of 12%(v/v) was added 10mg, 20mg, 30mg, 40mg, 50mg and 100mg of extract of *Phellinus linteus*(PL Ex.) and each mixture was dissolved with well stirring. The color, taste and precipitate of each portwine was compared with those of original 15 portwine.

The results are as follows.

20

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

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Preparation Example 6

To each 100ml of milk was added 5mg and 1,000mg of extract of *Phellinus linteus*(PL Ex.) and each mixture was dissolved with well stirring and filled in each bottle of 100ml and pasteurized. The color, 5 taste and precipitate of each milk was compared with those of original milk.

The results are as follows.

| | PL Ex. | 5mg | 1,000mg |
|----|--------|-----------|------------------------------|
| 10 | Color | no change | no change |
| | Taste | " | somewhat thick and tasteless |
| | PPT | non | non |

Preparation Example 7

15 To each solution of 1.0g of each dried granulized coffee extract in each 80ml of hot water was added 10mg, 20mg, 30mg, 40mg, 50mg and 100mg of extract of *Phellinus linteus*(PL Ex.) and each mixture was dissolved with well stirring to make each black coffee. The color, taste and precipitate of each black coffee was compared with those of original black coffee.
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The results are as follows.

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

5

Preparation Example 8

To each solution of 2.0g of dried granulized coffee extract in each 200ml of water was added 10mg, 20mg, 30mg, 40mg, 50mg and 100mg 10 of extract of *Phellinus linteus* (PL Ex.) and each mixture was dissolved with well stirring, filled in each can of 200ml, sealed and sterilized in a conventional preparation method of canned coffee. The color, taste and precipitate of each canned coffee was compared with those of original can coffee.

15

The results are as follows.

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

20

Preparation Example 9

To each 1.0g of dried granulized coffee extract was added 10mg,

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20mg, 30mg, 40mg, 50mg and 100mg of extract of *Phellinus linteus*(PL Ex.) and each mixture was well mixed with stirring and was sealed in each paper box. They are stored for 6 months at ambient temperature. Each box was dissolved in each 80ml of hot water and the color, taste 5 and precipitate of each coffee was compared with those of original coffee.

The results are as follows.

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

Preparation Example 10

15 To each solution of 2.0g of dried granulized green tea extract in each 200ml of water was added 10mg, 50mg, 100mg, 500mg, 1,000mg and 3,000mg of extract of *Phellinus linteus*(PL Ex.) and each mixture was dissolved with well stirring, filled in each can of 200ml, sealed and sterilized in a conventional preparation method of canned tea. The color, 20 taste and precipitate of each can tea was compared with those of original canned green tea.

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The results are as follows.

| PL Ex. | 10mg | 50mg | 100mg | 500mg | 1,000mg | 3,000mg |
|--------|-----------|-----------|-----------|-----------|------------------------------|------------------------------|
| Color | no change | no change | no change | no change | somewhat thick and tasteless | somewhat thick and tasteless |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

Preparation Example 11

To each 1.0g of dried granulized red tea extract was added 10mg, 20mg, 30mg, 40mg, 50mg and 100mg of extract of *Phellinus linteus*(PL Ex.) and each mixture was well mixed with stirring and was sealed in each paper box. They were stored for 6 months at ambient temperature. Each box was dissolved in 80ml of hot water and the color, taste and precipitate of each red tea was compared with those of original red tea.

15

The results are as follows.

| PL Ex. | 10mg | 20mg | 30mg | 40mg | 50mg | 100mg |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
| Color | no change |
| Taste | " | " | " | " | " | " |
| PPT | non | non | non | non | non | non |

The present extract of *Phellinus linteus*(PL Ex.) can be mixed with

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powder of dried coffee extract or dried granulized coffee extract and the mixture can be dissolved in hot water; or can be dissolved with coffee extract to prepare an instant coffee solution.

5 The present extract of *Phellinus linteus*(PL Ex.) can be mixed with powder of dried tea extract or dried granulized tea extract and the mixture can be dissolved in hot water; or can be dissolved with tea extract to prepare an instant tea solution.

10 Any other kinds of fruit juice of the present invention than orange juice can be prepared in accordance with the Example 1. It is evident that they come under the scope of the present invention.

15

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What we claim is:

1. A health beverage containing extract of *Phellinus linteus* as main ingredient and other ingredients commonly used in common beverage prepared by conventional preparing method of beverage with extract of 5 *Phellinus linteus* and other ingredients used in common beverage.
2. A health beverage of the claim 1 containing 1.0 – 5,000mg of extract of *Phellinus linteus* as main ingredient in 100ml of the healthy beverage and other ingredients commonly used in common beverage prepared by conventional preparing method of beverage with extract of 10 *Phellinus linteus* and other ingredients used in common beverage.
3. A health beverage of the claim 1 containing extract of *Phellinus linteus* as main ingredient and other ingredients commonly used in common beverage wherein other ingredients commonly used in common beverage are selected from the group consisting of juice, flavor 15 beverage, milk, alcoholic beverage, cola, coffee and tea.
4. A health beverage of the claim 1 containing extract of *Phellinus linteus* as main ingredient and other ingredients commonly used in coffee beverage wherein coffee beverage is an instant coffee drink containing extract of *Phellinus linteus* as main ingredient and other ingredients 20 commonly used in instant coffee drink; or a dried coffee mixture containing extract of *Phellinus linteus* as main ingredient and dried powder or dried granulated coffee extract in mixed state and when

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drinking the coffee beverage, the coffee mixture is diluted in water.

5. A Manufacturing method of health beverage containing extract of *Phellinus linteus* as main ingredient and other ingredients commonly used in common beverage prepared by conventional preparing method of 5 beverage with extract of *Phellinus linteus* and other ingredients used in common beverage.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR 97/00246

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁶: A 23 L 2/38; A 23 C 9/00; A 23 F 5/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁶: A 23 L 1/28, 2/00, 2/38; A 23 C 9/00; A 23 F 5/14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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